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(11) Publication number: 0 512 334 A3

(12)

## EUROPEAN PATENT APPLICATION

(21) Application number: 92106989.4

(51) Int. Cl.<sup>5</sup>: C12Q 1/68, //C12Q1/70

(22) Date of filing: 24.04.92

(30) Priority: 02.05.91 US 695201

(43) Date of publication of application:  
11.11.92 Bulletin 92/46

(84) Designated Contracting States:  
AT BE CH DE DK ES FR GB IT LI NL SE

(98) Date of deferred publication of the search report:  
03.03.93 Bulletin 93/09

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(54) Methods for detecting a target nucleic acid in a sample.

(57) This invention relates to improved methods for nucleic acid detection using amplification methods such as the polymerase chain reaction (PCR). More specifically, the invention provides methods for simultaneous amplification and detection to enhance the speed and accuracy of prior methods. The methods involve the introduction of detectable DNA binding agents into the amplification reaction, which agents produce a detectable signal that is enhanced upon binding double-stranded DNA. In a preferred embodiment, the binding agent is a fluorescent dye. The methods also provide means for monitoring the increase in product DNA during an amplification reaction.

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	WO-A-9 015 881 (CIS BIO INTERNATIONAL) * page 24 - page 28 *	1	C12Q1/68 //C12Q1/70
A	---	2-11, 14-24	
Y	NUCLEIC ACIDS RESEARCH vol. 19, no. 1, 11 January 1991, LONDON pages 109 - 116 S.T.ISAACS ET AL. 'Post-PCR sterilization: development and application to an HIV-1 diagnostic assay' * page 112 *	1	
A	---	2-5	
D,A	JOURNAL OF MOLECULAR BIOLOGY vol. 78, no. 4, 25 August 1973, NEW YORK US pages 703 - 714 J.P.RICHARDSON 'Mechanism of ethidium bromide inhibition of RNA polymerase' * abstract * * page 707; figure 2 *	1	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
A	EP-A-0 200 362 (CETUS CORPORATION) * column 29, line 17 - line 22 * * column 40, line 10 - column 41, line 3 *	1-17	C12Q
A	BE-A-1 000 572 (M.J.BLOCK) * page 17, line 33 - page 19, line 3 *	1,12,13	
A	DATABASE WPIL Week 8651, Derwent Publications Ltd., London, GB; AN 86-338273 & SU-A-1 231 077 (APPL MICROBIOL RES) 15 May 1986 * abstract *	1-11, 14-16, 19,20	
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The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 14 DECEMBER 1992	Examiner DE KOK A.J.
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure F : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : member of the same patent family, corresponding document			

EPO FORM 1500 CL.51 (P0401)



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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Character of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
P,X	BIOTECHNOLOGY vol. 10, no. 4, April 1992, NEW YORK US pages 413 - 417 R.HIGUCHI ET AL. 'Simultaneous amplification and detection of specific DNA sequences' * the whole document *	1-24	
E	EP-A-0 487 218 (TOSOH CORPORATION) * the whole document *	1-21,23, 24	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
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